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Amebic Encephalitis & Keratitis

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Free-living amoebae are unicellular protozoa common to most soil and aquatic environments. Of the many hundreds of species of free-living amoebae, only the following are known to cause diseases in humans:

1. members of the genus Acanthamoeba
2. *Naegleria fowleri*
3. *Balamuthia mandrillaris*

Acanthamoeba and *B mandrillaris* trophozoites may be recognized by the presence of slender, spine-like processes. When rounded, the cells measure 25 to 40 µm in diameter. The finely granular cytoplasm, as a rule, contains a single nucleus with a large, dense central nucleolus surrounded by a nuclear clear zone. Water and digestive vacuoles are usually visible in the cytoplasm. The double-walled cysts are generally polygonal, spherical, or star-shaped, 15 to 20 µm in diameter, with a nucleus containing a large dense central nucleolus surrounded by a clear nuclear halo. The smooth inner wall of the cyst contacts the wrinkled outer wall at a number of points, forming pores, opercula, or ostioles. Reproduction is by binary fission of the trophozoites.

Ecology

Acanthamoeba is a genus of environmental free-living amoebae found in most soil and water habitats. The genus contains numerous species, of which *A. polyphaga*, *A. castellanii* and *A. culbertsoni* have been identified most frequently as causing human disease. Acanthamoeba have been isolated from natural and artificial waters, chlorinated swimming pools and the atmosphere. Acanthamoeba is an aerobic organism but cysts have been isolated from anaerobic material such as feces and sewage. The trophozoites are killed by saline concentrations greater than 1%, although the more environmentally robust cysts have been isolated from marine environments. Acanthamoeba numbers in freshwater habitats depend on temperature and bacterial food source. Acanthamoeba cysts have been isolated in marine sites, particularly those associated with sewage and waste effluent outlets.

Balamuthia mandrillaris: In 1990, Visvesvara and colleagues described cases of fatal encephalitis in humans and other primates due to a previously undescribed free-living amoeba. They were found to be sufficiently distinct to be described as a new genus and species, *Balamuthia mandrillaris* (Visvesvara et al). Using antiserum to the organism, the investigators were able to demonstrate that certain cases of GAE (Granulomatous Amoebic Encephalitis) attributed to Acanthamoeba were in fact caused by *B.mandrillaris*.

Naegleria: There are several species of Naegleria, but *Naegleria fowleri* is the only species that has been shown to cause disease in humans. Naegleria is commonly found in warm freshwater and soil around the world. This can include:

- lakes and rivers
- naturally hot water such as hot springs
- poorly maintained and under-chlorinated or unchlorinated swimming pools
- warm water discharge from industrial plants
- soil, however water is the only known source of human infection.

They grow best in warm water, especially between 25°C and 40°C. Any water body that seasonally exceeds 30°C or continually exceeds 25°C can support the growth of *N.fowleri*.

Naegleria infects people by entering the body when water containing the amoeba goes up the nose. This may occur:

- when people swim, dive or fall into warm freshwater containing *Naegleria*.
- following domestic bathing, for example when young children fall or slip in a bath of water containing *Naegleria*.

Naegleria infections do not occur as a result of drinking contaminated water, swimming in the sea, or from swimming in a properly cleaned, maintained and chlorinated swimming pool.

The organism was first identified in South Australia during the 1960s. A number of cases of infection occurred in towns served by unchlorinated water delivered through long above-ground pipelines. About half the cases had swum in warm freshwater, and the other half had sniffed or squirted water from the town supply into their noses. There have been no cases in South Australia since 1981, with chloramination of the water supply (a water treatment that ensures good residual levels of chlorine), and a public education campaign.

The amoebae travel up the nose to the base of the brain where they infect brain tissue. The risk of infection is extremely small. Children and young adults appear to be more susceptible to infection than adults.

Persistence in the environment

N.fowleri cannot survive in water that is clean, cool and chlorinated. Free chlorine or chloramines at 0.5mg/L or higher will control *N.fowleri*, provided that the disinfectant persists through the water supply system.

Infective cysts do persist in dust and aerosols.

Incidence /Disease risk

GAE due to Acanthamoeba is extremely rare, with only 60 cases reported worldwide.

Although *N.fowleri* can be commonly found in the environment, infection is rare. Cases of Naegleria meningoencephalitis have been recorded in South Australia, Western Australia, Queensland and New South Wales, and in many countries throughout the world. The risk of *Naegleria fowleri* infection is very low. There have been 30 reported infections in the U.S. in the ten years from 2000 to 2009, despite millions of recreational water exposures each year. By comparison, in the ten years from 1996 to 2005, there were over 36,000 drowning deaths in the U.S.

In the United States, in the ten years from 1998 to 2007, 33 infections were reported. Thirty-one had contact with recreational water and two had contact with water from a geothermal (naturally hot) water supply. It is estimated that the risk from recreational water activities (such as swimming/diving/waterskiing) in potentially contaminated freshwater in the U.S., is five cases of *N.fowleri* infection for every billion episodes of recreational water activity.

Approximately 85 cases of *B. mandrillaris* encephalitis have been described worldwide, with some 50% coming from the U.S.. At least ten cases have occurred in patients with human immunodeficiency virus (HIV). Other cases have been identified from Argentina, Australia, Canada, Czechoslovakia, Japan, Mexico and Peru.

Pathogenesis

Free-living amebas have been isolated from human throats, suggesting that they are generally harmless in healthy individuals. *Acanthamoeba* spp usually act as opportunistic pathogens, taking advantage of a loss of metabolic, physiologic, or immunologic integrity by the host. Among the most common factors predisposing an individual to Acanthamoeba infection are immunosuppressive therapy, treatment with broad-spectrum

antibiotics, diabetes mellitus, various cancers, malnutrition, pregnancy, acquired immune deficiency syndrome (AIDS), and chronic alcoholism. Surgical trauma, burns, wounds, and radiation therapy can also promote infection.

The primary focus of infection for opportunistic Acanthamoeba is usually the lower respiratory tract or skin. The amebas may enter the respiratory tract by the inhalation of aerosols or dust containing cysts. Spread to the CNS is apparently hematogenous. The cerebral hemispheres in GAE may be edematous, with focal cortical softening, hemorrhage and abscesses. Uncal notching and cerebellar herniation may be present. Foci of hemorrhagic necrosis may be seen in the basal ganglia, midbrain, brainstem, and cerebellum. The histopathologic changes consist of a chronic granulomatous encephalitis with multinucleated giant cells, mainly in the posterior fossa structures, basal ganglia and cerebellum. Trophozoites and cysts may be found in the lesions.

Except in the case of amebic keratitis, the defenses of a healthy host seem sufficient to prevent Acanthamoeba or ***B. mandrillaris*** infection. Patients who contract GAE usually have impaired humoral and/or cell-mediated immunity. However, there are reports of patients with no demonstrable underlying disease or predisposing factor.

Semiology

Acanthamoeba

Species of Acanthamoeba that are pathogenic to humans can cause two clinically distinct diseases:

1. Granulomatous amoebic encephalitis (GAE)
2. Keratitis (inflammation of the cornea)

A. polyphaga and *A. castellanii* are most frequently reported as causing keratitis; *A. culbertsoni*is most frequently reported as causing GAE

Granulomatous Amoebic Encephalitis is a chronic disease of the immunosuppressed (chemotherapy, AIDS, drug or alcoholic abuse) host. GAE is subacute or chronic and invariably fatal. Symptoms include fever, headache, seizures, meningitis and visual abnormalities. The route of infection in GAE is unclear, although invasion of the brain may result from the blood following a primary infection elsewhere, possibly the skin or lungs. The precise source of such infections is unknown because of the almost ubiquitous presence of Acanthamoeba in the environment.

Acanthamoeba keratitis affects previously healthy persons and is a severe and potentially blinding infection of the cornea. Untreated acanthamoeba keratitis can lead to permanent blindness. Unilateral infection is the more common form. The disease is characterized by intense pain and ring-shaped infiltrates in the corneal stroma. Contact lens wearers are most at risk from the infection and account for approximately 90% of reported cases. Poor contact lens hygiene practices (notably ignoring recommended cleaning and disinfection procedures and rinsing or storing of lenses in tap water or non-sterile saline solutions) are recognized risk factors, although the wearing of contact lenses while swimming or participating in other water sports may also be a risk factor. In noncontact lens related keratitis, infection arises from trauma to the eye and contamination with environmental matter such as soil and water.

Acanthamoebic pneumonitis and dermatitis, characterized by the presence of cysts and trophozoites in alveoli or in multiple nodules or ulcerations of the skin, are opportunistic diseases that usually affect immunosuppressed or debilitated individuals. In acanthamoebic pneumonitis, chest radiographs may show areas of consolidation

Naegleria causes a very rare infection of the brain and brain coverings called primary amoebic meningoencephalitis (PAM). Even with treatment, most people with *Naegleria fowleri* infection die.

In humans, *N. fowleri* can invade the central nervous system via the nose, more specifically through the olfactory mucosa and cribriform plate of the nasal tissues. The penetration initially results in significant necrosis of and hemorrhaging in the olfactory bulbs. From there, the amoebae climbs along nerve fibers

through the floor of the cranium via the cribriform plate and into the brain. It begins to consume cells of the brain piecemeal by means of a unique sucking apparatus extended from their cell surface. It then becomes pathogenic, causing primary amoebic meningoencephalitis (PAM or PAME). PAM is a syndrome affecting the central nervous system. PAM usually occurs in healthy children or young adults with no prior history of immune compromise who have recently been exposed to bodies of fresh water.

Onset symptoms of infection start one to 14 days after exposure. The initial symptoms include, but are not limited to changes in taste and smell, also headache, fever, nausea, vomiting, and stiff neck. Secondary symptoms include confusion, hallucinations, lack of attention, ataxia, and seizures. After the start of symptoms, the disease progresses rapidly in three to seven days, with death occurring from seven to 14 days.

Like Acanthamoeba GAE, ***B. mandrillaris*** **encephalitis** is largely a disease of the immunocompromised host and infects either sex, and any age; however, cases are being recognized in persons with no underlying immunosuppression and with no history of contact or swimming in water. The clinical course of the disease in humans ranges from 14 days to six months, with a mean of 75 days. Infection is invariably fatal. Clinical symptoms and histopathological findings are similar to those seen in GAE, and cysts are also found in the tissues.

Diagnostic

Timely diagnosis remains a very significant impediment to the successful treatment of infection, as most cases have only been discovered post-mortem.

In many cases, GAE is not diagnosed until after or, at best, shortly before death. Immunosuppression or other predisposing factors may provide important clues. The differential diagnosis includes space-occupying lesions such as tumors, abscesses, and even infarcts, as well as tuberculoma or fungal infection. Computed tomography and magnetic resonance imaging of the brain are important diagnostic tests, as is examination of cerebrospinal fluid and brain biopsy specimens. The diagnosis usually is made after examination of brain tissue with light a microscope.

Amebic “dermatitis” is often diagnosed by microscopic examination of a skin biopsy. Both trophozoites and cysts are usually visible.

In the case of amebic keratitis, scrapings of the corneal ulceration and biopsy specimens may contain amebic trophozoites and cysts. Both light and electron microscopy may be useful. Amebic cysts in the corneal stroma may be demonstrated by staining with hematoxylin and eosin, trichrome, calcofluor-white, or immunofluorescence techniques.

Culture

N. fowleri can be grown in several kinds of liquid axenic media or on non-nutrient agar plates coated with bacteria. *Escherichia coli* can be used to overlay the non-nutrient agar plate and a drop of CSF sediment added to it. Plates are then incubated at 37°C and checked daily for clearing of the agar in thin tracks, which indicate that the trophozoites have fed on the bacteria. Detection in water is performed by centrifuging a water sample with *E.coli* added, and then applying the pellet to a non-nutrient agar plate. After several days the plate is microscopically inspected and Naegleria cysts are identified by their morphology. Final confirmation of the species' identity can be performed by various molecular or biochemical methods. Confirmation of Naegleria presence can be done by so called flagellation test, when the organism is exposed to a hypotonic environment (distilled water). Naegleria in contrast to other amoebae differentiates within two hours into the flagellar state. Pathogenicity can be further confirmed by exposition to high temperature (42°C): *N.fowleri* is able to grow at this temperature, but the non-pathogenic *N. gruberi* is not.

Unlike *N. fowleri* and Acanthamoeba, *B. mandrillaris* does not grow on the standard medium for isolating free-living amoebae: plain agar seeded with the bacterium *E. coli. B. mandrillaris* has been cultured from only a few cases of infection using mammalian tissue culture cell lines. As a consequence of the difficulties in growing the organism, there have been no reports of the isolation of *B. mandrillaris* from water or other environmental samples

Environmental sampling:

Recognition of *N.fowleri* in water requires specialized testing. Testing to identify the amoeba is not done routinely and is expensive. It should be assumed that any warm freshwater body as described above could contain *N.fowleri*.

Treatment

Amphotericin B is effective against *N. fowleri* in vitro, but the prognosis remains bleak for those that contract PAM, and survival remains less than 1%. On the basis of the in vitro evidence alone, the Centers for Disease Control and Prevention (CDC) currently recommends treatment with Amphotericin B for primary amebic meningoencephalitis, but there is no evidence that this treatment affects outcome. Treatment combining miconazole, sulfadiazine, and tetracycline has shown limited success only when administered early in the course of an infection.

While miltefosine had therapeuthic effects during an in vivo study in mice, Chlorpromazine showed to be the most effective substance - the authors concluded: "Chlorpromazine had the best therapeutic activity against *N. fowleri* in vitro and in vivo. Therefore, it may be a more useful therapeutic agent for the treatment of PAME than amphotericin B."

Prevention

N. fowleri is found in many warm freshwater lakes and rivers in the United States, particularly in southern tier states. It is likely that a low risk of *N. fowleri* infection will always exist with recreational use of warm freshwater lakes, rivers, and hot springs. The low number of infections makes it difficult to know why a few people have been infected compared to the millions of other people using the same or similar waters across the U.S. The only certain way to prevent a *N. fowleri* infection is to refrain from water-related activities in warm, untreated, or poorly-treated water. If you do plan to take part in water-related activities some measures that might reduce risk include:

Personal actions to prevent infection:

- Avoid jumping or diving into bodies of warm fresh water or thermal pools
- Keep head above water in spas, thermal pools and warm fresh water bodies
- Empty and clean small collapsible wading pools and let them dry in the sun after each use
- Ensure swimming pools and spas are adequately chlorinated and well maintained
- Flush stagnant water from hoses before allowing children to play with hoses or sprinklers if using unchlorinated water:
- Don't allow water to go up your nose when bathing, showering or washing your face
- Hold the nose shut or use nose clips when taking part in water-related activities in bodies of warm freshwater
- Supervise children playing with hoses or sprinklers and teach them not to squirt water up their nose
- Avoid digging in or stirring up the sediment while taking part in water-related activities in shallow, warm freshwater areas.
- Potentially contaminated water should not be used for any form of nasal irrigation or nasal lavage including Neti (an Ayurvedic practice of nasal cleansing).

Private wells

N.fowleri has been identified where bore water is rested in above-ground dams then piped over distances in above-ground pipes to private homes. The presence of *N.fowleri* will vary with ambient temperature, the distance water is piped, and the length of time the water is at temperatures favorable to the amoeba while in storage and pipework. This length of time may be related to the rate of water use. In such circumstances, measures to prevent infection should be observed. Seek specialist advice regarding the pros and cons of water treatment processes (e.g. chlorination, chloramination filtration, UV treatment).

Posting warning signs around recreational water bodies is not useful

Recreational water users should assume that there is always a low level of risk whenever they enter warm freshwater (for example, when swimming, diving, or waterskiing) in southern-tier states. Posting signs is unlikely to be an effective way to prevent infections. This is because the location and number of amebae in the water can vary over time. In addition, posted signs might create a misconception that bodies of water without signs are *N.a fowleri*-free.